Epigenetic Control of NRF2-Directed Cellular Antioxidant Status in Dictating Life-Death Decisions

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Suppression of transcription factor NRF2 by ARF highlights epigenetic control of cellular antioxidant status in dictating life-death decisions

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Abstract/summary

Chen et al. (2017) describe that stimulation by ARF of p53-independent cell death requires down-regulation of NRF2-target genes through a mechanism that involves physical association between ARF and NRF2, and which results in loss of acetylation of NRF2 by CBP/p300.

Main text

Upon its activation, the tumor suppressor protein ARF (Alternative Reading Frame, also called $p14^{ARF}$ in human and $p19^{ARF}$ in mouse) can trigger apoptosis. The cap'n'collar (CNC) basic-region leucine zipper (bZIP) transcription factor NF-E2 p45-related factor 2 (NRF2, encoded by *NFE2L2*) is a master regulator of redox homeostasis that protects against apoptosis. It is therefore apparent that NRF2 potentially antagonizes ARF. Remarkably, Wei Gu and colleagues show in a recent issue of *Molecular Cell* that ARF physically interacts with NRF2 and suppresses the expression of NRF2-target genes, thereby allowing cell death to proceed (Chen et al., 2017).

The activity of ARF is increased by various stress pathways including hyper-proliferation caused by the aberrant expression of oncogenes (Ko et al., 2016). Under such circumstances, ARF triggers tumor suppressor responses, including apoptosis and ferroptosis, through activation of both p53-dependent and p53-independent pathways (see papers cited in Chen et al., 2017). Relatively little is known about p53-independent mechanisms by which ARF inhibits tumor growth. Because expression of the cystine/glutamate antiporter SLC7A11 is strongly negatively associated with susceptibility to ferroptosis stimulated by reactive oxygen species (ROS), and because *SLC7A11* is regulated by NRF2 through antioxidant response element (ARE) sequences in its gene promoter, Chen et al. (2017) explored whether

ARF might stimulate ferroptosis in a p53-independent manner by antagonizing NRF2, and thus down-regulating *SLC7A11*. Using co-immunoprecipitation assays, these workers demonstrated that the *N*-terminal half of ARF physically interacts with the *C*-terminal Neh1 and Neh3 domains of NRF2 (see Figure 1A for NRF2 structure). They also found that induction of ARF in a human osteosarcoma p53-null cell line that possessed a stably transfected tetracyclin-regulated ARF-expression plasmid resulted in a markedly decreased expression of *SLC7A11*, along with other NRF2-target genes, whereas knockdown of ARF increased expression of these genes. As anticipated, the down-regulation of *SLC7A11* following ARF induction in human non-small cell lung carcinoma p53-null cells resulted in substantially increased cell death via both ferroptosis and apoptosis when they were treated with the ROS-generating agent *tert*-butyl hydroperoxide. Moreover, this could be prevented by ectopic overexpression of NRF2. Most surprisingly however, Chen et al (2017) reported that the inverse relationship between ARF abundance and NRF2 activity is because ARF inhibits acetylation of the transcription factor.

The discovery that NRF2 activity can be blunted as a consequence of blocking its acetylation is unexpected because NRF2 is principally regulated at the protein stability level (reviewed in, Tebay et al., 2015). In fact, few researchers have reported acetylation of NRF2. Using mass spectrometry, Donna Zhang and colleagues provided the first evidence that ectopically-expressed NRF2 can be acetylated by CREB-binding protein (CBP)/p300 at Lys residues within its Neh1 domain, and that this posttranslational modification enhances DNA-binding and gene transactivation activities of NRF2 (Sun et al., 2009). These workers also demonstrated that deletion of the Neh4 and Neh5 domains largely abolished the ability of CBP/p300 to acetylate ectopic NRF2, which is consistent with the notion that NRF2 recruits CBP/p300 to the regulatory regions of ARE-containing genes via its Neh4 and Neh5 domains (Sekine et al., 2015). Subsequently, the late Ifeanyi Arinze and colleagues confirmed that

CBP/p300 can acetylate ectopic NRF2, and proposed that acetylation of Lys-588 and Lys-591 in mouse Nrf2 (equivalent to Lys-596 and Lys-599 in human NRF2) increases transactivation activity of the CNC-bZIP factor to the greatest extent (Kawai et al., 2011). Besides CBP/p300, the histone acetyltransferase MOF can acetylate NRF2, and knockdown of MOF in human A549 cells has been reported to decrease expression of NRF2-target genes (Chen et al., 2014).

The above data suggest NRF2 recruits CBP/p300 to ARE sequences in DNA via its Neh4 and Neh5 domains, whereupon CBP/p300 acetylates the Neh1 and Neh3 domains of NRF2, and/or surrounding histones, thereby increasing ARE-driven gene expression (Figure 1B). Importantly, the Neh1 and Neh3 domains of NRF2 have at least three functions: i) hetero-dimerization with a small musculoaponeurotic fibrosarcoma oncogene homolog (sMAF) bZIP transcription factor; ii) binding to ARE sequences in target genes; iii) interaction with the ATP-dependent chromatin remodeling enzyme chromodomain helicase DNA-binding protein-6 (CHD6) through its sixteen *C*-terminal residues (Nioi et al., 2005). Any, or all, of these functions might be affected by acetylation. Therefore, ARF may suppress NRF2 activity because it inhibits acetylation and/or because it sterically impairs hetero-dimerization of NRF2 with a sMAF protein or the interaction of NRF2 with CHD6 (Figure 1C). In addition, ARF might also alter the ability of NRF2 to bind to the mediator subunit 16 (MED16), which in turn stimulates recruitment of RNA polymerase II to ARE-containing genes (Sekine et al., 2016), but this possibility has not been addressed.

Consistent with the view that NRF2 is positively regulated by acetylation, sirtuin-1 has been described to de-acetylate NRF2 and decrease gene transactivation, and that this can be exacerbated by resveratrol, an activator of sirtuin-1 (Kawai et al., 2011). Conversely, histone deacetylase (HDAC) inhibitors stimulate NRF2 activity (McMahon et al., 2014) but it is not known whether this is mediated by increasing NRF2 acetylation or by increasing the

acetylation of histones associated with ARE-containing gene promoters/enhancers. In a similar vein, glucocorticoid receptor signaling can repress NRF2 by recruitment of HDAC to their binding sites in the promoters of NRF2-target genes, but whereas it is clear that this decreases acetylation of histone H3 Lys-27 and CBP/p300 recruitment (Alam et al., 2017), the possibility that the NRF2 acetylation status might be also altered cannot be excluded.

A number of important issues require to be addressed. Firstly, in addition to *SLC7A11*, NRF2 regulates the basal and/or inducible expression of possibly 500 ARE-driven genes (Tebay et al., 2015), and it is not known to what extent the down-regulation of these other genes by ARF also contributes to the suppression of ferroptosis and apoptosis. Secondly, it is unclear whether the stimulation of cell death by ARF that proceeds upon its antagonism of NRF2 involves depletion of antioxidants along with a concomitant increase in ROS. Thirdly, the *in vivo* importance of acetylation of NRF2 versus acetylation of chromatin surrounding ARE-containing gene promoters/enhancers has yet to be established. Fourthly, whilst it seems likely that upon binding ARE sequences NRF2 first recruits CBP/p300, the subsequent order of recruitment of CHD6 and MED16, and other factors, has yet to be established. Fifthly, it is not known if the ability of ARF to repress NRF2 involves re-localization of the CNC-bZIP transcription factor to the nucleolus, as this is a mechanism by which ARF inhibits MDM-2 activity.

Selected reading

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Figure 1. Model for antagonism of NRF2 activity by ARF under oncogenic stress conditions

(A) Cartoon of NRF2 showing the relative positions of the Neh4 (amino acids 99-145) and Neh5 (amino acids 183-201) transactivation domains, the Neh1 (amino acids 435-562) CNC-bZIP DNA-binding and sMAF protein dimerization domain, and the *C*-terminal Neh3 (amino acids 563-605) CHD6 interaction domain; note, amino acid numbering is based on the human NRF2 protein. The positions of Lys residues in the Neh1 and Neh3 domains that are acetylated by CBP/p300 are depicted as yellow circles. **(B)** Under oxidative stress conditions, NRF2 (in blue, towards left-hand side of cartoon) is stabilized and accumulates in the nucleus where it binds ARE sequences in target genes as a heterodimer with a sMAF protein (brown circle). Thereafter, NRF2 recruits various proteins to these sites to allow gene transcription to occur, though the specific order of events is uncertain. In particular, NRF2 recruits CBP/p300 to ARE sequences, via its Neh4 and Neh5 domains, which allows CBP/p300 to acetylate NRF2 in its Neh1 and Neh3 domains (depicted by yellow circles), and presumably the recruitment of CBP/p300 by NRF2 also increases acetylation of histone H3 Lys-27 (H3K27) in chromatin around ARE sequences that is associated with active enhancers to permit transcription. In addition, NRF2 binds CHD6 (pink oval, bottom left-hand side) through its Neh3 domain, which likely increases the accessibility of DNA surrounding the ARE sequence. Furthermore, NRF2 binds MED16 through its Neh4 and Neh5 domains, and this leads to recruitment of the mediator complex that allows assembly of the preinitiation complex along with RNA polymerase II (shown in the center of the cartoon). Thus, during oxidative stress the increased binding of NRF2-sMAF heterodimers to ARE sequences in target genes triggers a cascade of events that ultimately leads to the induction of antioxidant genes such as *SLC7A11, GCLC, GCLM, GPX2* and *TXNRD1*, which act to suppress ROS levels and diminish the likelihood of apoptosis and ferroptosis. **(C)** Under oncogene stress conditions, intracellular levels of ARF (green oval, at bottom left-hand side) accumulate as a consequence of gene induction and protein stabilization. The resulting increase in ARF

protein allows formation of a complex between ARF and NRF2, which arises through interactions between the *N*-terminal region of ARF and the Neh1 and Neh3 domains of NRF2. Binding of ARF to NRF2 blunts acetylation of NRF2 by CBP/p300, and it may also blunt acetylation of H3K27 in the vicinity of ARE sequences. Also, ARF probably sterically inhibits recruitment of CHD6 to NRF2. Through these varied mechanisms, ARF attenuates the activity of NRF2 resulting in the down-regulation of its target genes. Presumably down-regulation of the ARE-gene battery results in diminished antioxidant status and higher ROS levels, which renders cells more sensitive to apoptosis and ferroptosis. (Image created by Rumen V. Kostov, University of Dundee).